# The Effect of Blood Contamination on the Retrograde Sealing Ability of Biodentine®: A Micro-CT Analysis

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# DEDICATION

To my amazing wife, Diane, for her endless support and encouragement. There is absolutely no possible way this happened without you. To my kids, for your welcoming love every day. To my mom and dad, for encouraging lifelong learning and showing me the importance of work hard.

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# INTRODUCTION

The history of dental materials in endodontics has evolved over the course of several centuries. This evolution can be seen from the early uses of gold, gutta-percha, amalgam, resilon, and now with modern materials like calcium silicates. With each new material, the goals remain the same: to seal the root canal space to prevent contamination from microorganisms and to prevent inflammation at the tissue/material interface. It is important that proper testing is completed to see how the material adapts to the environment in which it will be placed. We have seen in the history that there have been materials that have been successful and continue to be used today or materials that have shown toxicity to the human body over time.

When we start to examine the different materials that have been used in apical surgeries, we also see the evolution from gutta-percha, amalgam, IRM, Super EBA to bioceramic materials such as Mineral Trioxide Aggregate (MTA). With each newly developed material, advancements are sought after to create a material with the ideal characteristics for a myriad of applications. These newer bioceramic materials have applications that allow a clinician to place these materials close to the pulp chamber, directly on an exposed pulp, perforation repairs, and in apical surgeries where the materials will come in contact with blood and cells important to the remineralization of the bone in that area.

Bioceramic type materials have undergone various studies to test their ideal characteristics. Many of these studies have been *in vitro* models that place

the material in an ideal setting to test for sealing ability, inflammation, response to different cell types, etc. These *in vitro* studies provide us with a basis for our clinical practice and still hold value due to the cost and difficulty to bring about some clinical trials. However, what happens when the clinical environment isn't ideal like the *in vitro* studies?

During apical surgery procedures, there will invariably be blood, inflammatory byproducts, oral fluids, saline for surgical use, etc. What affect do these fluids have on the sealing ability of our bioceramic retrofilling materials? Can we ensure that we are completely sealing the tooth after surgery if there is blood contamination? The aim of this study is to determine the effect blood contamination has on the sealing ability of a newer bioceramic material, Biodentine®, when it is used as a retrofilling material. The use of micro-computed tomography technology and a silver nitrate tracer served as a method to investigate the sealing ability into the retropreparation and the interface between the dentinal walls and retrograde filling material.

# LITERATURE REVIEW

One of the basic aims of endodontics is to prevent and treat apical periodontitis. The cause for apical periodontitis is the bacterial invasion of the root canal space (1,2) which then triggers a host response (3). Treatment of apical periodontitis is initially completed by way of a non-surgical root canal treatment. The purpose of endodontic treatment is to remove the etiology/bacteria from the canal system and seal the canal system as best as possible (4). Sealing the canal both apically and coronally to prevent a recontamination of the root canal system is of the utmost importance (5). Our obturation goals are accomplished when this sealing of the canal is completed and there is resolution of both the patient's symptoms and of the apical periodontitis. Radiographically, this resolution may take months to years (6,7). In certain instances, initial root canal therapy may not be successful and would require further treatment to remove the etiology, which may include either retreatment or apical surgery (8). When comparing the two treatment options, both have been shown to have very comparable outcomes and are fairly predictable (9,10).

## APICAL SURGERY

The apical surgery procedure itself requires multiple steps: flap reflection, osteotomy to expose the root apex, root end resection, root end preparation, root

end filling, and flap closure. Each step in this procedure has been studied in various ways and evolved over time. The use of magnification with illumination, CBCT imaging, modern instrumentation, and high quality root end filling materials have vastly improved surgical techniques and outcomes (11).

**Flap Reflection/Hemostatic Control**

In order to maintain visualization and a dry surgical field, flap reflection has been shown to be a key element to achieving this goal. There are several flap designs that can be used in each surgical situation, each with its own pros and cons. When possible, flaps should be full thickness and broader at the base of the flap (12). Main vasculature in the oral cavity runs parallel to the alveolar ridge in the vestibule so care should be taken to limit severing of these vessels (13). Blood contamination of the apical retropreparation is always a possibility when doing an apical surgery. The typical apical surgery has been shown to not include much more blood loss than a typical tooth extraction (14). Different measures can be taken to limit the amount of blood into the surgical site and to try and avoid contaminating the root end preparation. Prior planning with the use of anesthetic with double the epinephrine concentration (1:50,000 vs. 1:100,000 epinephrine) has been shown to limit the amount of bleeding present during surgeries involving the periodontal tissues (15). During the procedure itself, the clinician can use various hemostatic measures to limit bleeding. These measures may include: bone burnishing, racemic epinephrine incorporated cotton pellets,

ferric sulfate, cautery, colla-plug, gelfoam, and others (16). Even with these measures, hemostasis can be a difficult process and there is potential for blood contamination.

**Root End Resection**

Apical surgery requires the removal of the root apex for several reasons: removal of pathologic processes and soft tissue lesions at the apex, access to the root canal therapy/obturation material, removal of isthmus and anatomic variations, evaluate the root end for cracks or fractures, and others (11). Modern recommendations are for a resection level of at least 3mm (17). Such a resection level has the purpose of removing any apical ramifications and the lateral canals that are typically found in the apical third of roots (18). Each tooth type is different however, and the variation in anatomy may call for different resection levels. The mesiobuccal canal of maxillary molars, for example, has been shown to require a resection level of at least 3.6mm up to 5mm to remove isthmuses and allow proper dentin thickness so as to not crack the root during retropreparation (19,20). When an isthmus is present during an apical resection, our outcomes tend to suffer due to the complexity of the canal system and propensity for vertical root fractures in the future after retropreparation (21).

Historically, the angle of resection has also shown to be significant to apical surgery. Modern techniques recommend angles in the 0-10° bevel angle. An evaluation of the resection angle was completed by Gilheany *et al* and

revealed that microleakage decreased with a smaller bevel angle and a deeper retropreparation (22). If during apical surgery, the desired minimal bevel is unable to be accomplished, the recommendation is to ensure that your retropreparation is at a depth of at least 3mm (8). In a prospective clinical study using modern materials methods, cone beam computed tomography (CBCT) analysis of images taken before and 1 year after surgery, show an average resection angle of 17.7° ± 11.4° with no effect on treatment outcome among the teeth in the study (23).

**Root End Preparation**

Before the advent of ultrasonic tips for root end preparation, the use of the micro handpieces was how conventional root end preparations were completed. The disparity in size and ability to angulate these two instruments is vastly different. The micro handpiece technique resulted in overpreparation of the apex, preparations outside the long axis of the tooth, missed isthmuses, and other errors (24). Matsura developed a slot-end preparation which allowed for a more controlled technique of apical preparation but still required the removal of excess dentin at the apex (25).

Ultrasonic tips, which have been used for the past few decades, have improved upon the limitations of the micro handpiece for apical surgery use. No longer is it required to extend osteotomy sites to fit the head of the handpiece in the crypt for retropreparation. When combined with the use of magnification with

the dental operating microscope (DOM) and ultrasonic tips provide a conservative and accurate approach to endodontic surgery (26). Ultrasonic tips allow a more conservative osteotomy and provide a cleaner and deeper apical root end preparation (27,28). The use of ultrasonics has also been shown to not induce dentinal cracks or microfractures that may have been a worry when first introduced (29,30). The ultrasonic tips allow for a much deeper retropreparation compared to the micro handpiece and allows for more root end filling material to be delivered into the root end preparation (27).

### Root End Filling

Sealing the apical root after root end preparation has evolved over the course of the last century and different materials have been used (with varying degrees of success). Modern advancements in the use of the DOM (11), root end preparation with ultrasonic tips (24), and biocompatible root end filling materials, have led to an better overall prognosis for surgical intervention (26,31).

## ROOT END FILLING MATERIALS

### Traditional Materials

In *Color Atlas of Microsurgery in Endodontics* (2001), the authors discuss several ideal characteristics of a retrofilling material. Some of those characteristics include: seal off the root canal system, are biocompatible with the

surrounding periapical tissues, are able to be condensed, set up over time, can adhere to the tooth, are radiopaque, easy to use, do not shrink when set, do not stain, does not solubilize, and are non-staining (17). The evolution of materials over the past several decades have enabled more of these characteristics to be realized in a single material.

Some of the earliest materials used for root end filling materials were amalgam, zinc oxide eugenol materials such as IRM and Super EBA, glass ionomer cements, and resin bonded materials. These materials have different disadvantages that include needing a completely dry environment, retention form to stay in place, differences in leakage, toxicity to surrounding tissues, staining potential, and others (32). Even with several disadvantages, many of these materials had the possibility for success over time. Amalgam was shown to have a lower success rate (75%) when compared to IRM (91%) and Super EBA (95%) in a retrospective study comparing the materials (33). Long-term prognosis of amalgam retrofillings was also shown to be poor due to the lesions initially healing but then relapsing and being deemed failures after a few years (34). The objective still remains the same with any root end filling material: provide an antibacterial and fluid tight seal, to prevent recontamination after the apical portion of the root has been resected and the retrograde preparation completed (35). These traditional materials remained in favor until the early 1990’s when a newer material was introduced that provided a resurgence in endodontic materials and their applications as a retrofilling material in apical surgeries.

## Mineral Trioxide Aggregate (MTA)

MTA is a combination of a powder mix of type 1 Portland cement and radiopacifier. The material was introduced in the early 1990's and has been a focus of endodontic materials and research ever since. The composition of MTA itself is primarily dicalcium and tricalcium silicates, bismuth oxide which provides radiopacity, tricalcium aluminate, tetracalcium aluminoferrite, and gypsum (36). MTA currently has two formulations for endodontic application: white and grey. MTA has been shown to be less toxic when compared to amalgam, IRM and Super EBA (37); it has superior sealing ability compared to the traditional retrofilling materials (38); compressive strength (39); biocompatibility (40); as well as other important characteristics of an ideal filling material. Outcomes comparing the use of MTA to more traditional materials has shown that MTA may have a very similar or better outcome (41,42). MTA is a material that is well suited for apical surgery, perforation repairs, and vital pulp therapy. MTA has been the gold standard for retrofillings for a long time and in a recent 10 year retrospective study, if the tooth was healed at the one year recall, it had an 86.8% of being present at the 10 year recall (43). However, due to the longer setting time, wash-out potential, tooth discoloration, and the handling properties of MTA, new formulations of calcium-silicate based materials have been developed to try and improve upon the limitations of MTA.

## Endosequence® BC Root Repair Material

To facilitate the ease of use and application, Endosequence® BC Root Repair Material (BC Putty) (Brasseler, Savannah, GA, USA) is packaged as a premixed product. It is primarily composed of tricalcium and dicalcium silicates, zirconium oxide, tantalum pentoxide and calcium sulfate anhydrate. The product is dispensed via a syringe and the second generation Fast Set Putty™ has a manufactured setting time of 20 minutes with full set at 4 hours (44). Though BC Putty has similar components to MTA, it differs in the radiopacifier used and therefore BC Putty has more color stability and less staining potential (44). There is limited cytotoxicity with BC Putty and biocompatibility is similar to that of MTA (45). The material has also been shown to have antifungal (46) and antibacterial properties (47), similar to MTA. In a bacterial leakage model, both MTA and BC Putty showed similar resilience to bacteria in and out of the canal after retrograde filling (48). Overall success rates have been reported as similar to MTA when used as a retrofilling material (49,50). BC Putty can be seen as a suitable material for retrofilling, perforation repairs, and vital pulp therapy since both materials show similar responses to human tissues.

## Biodentine®

Another material that has been invented in the last decade has been Biodentine® (Septodont USA, Lancaster, PA). This material comes as a separate powder and liquid and requires a 30 second mixing in a capsule by a

triturator. The powder consists of tricalcium and dicalcium silicates, calcium carbonate, zirconium oxide and iron oxide. The liquid component contains calcium chloride and a hydrosoluble polymer. The calcium chloride acts as a setting agent which creates a lower initial setting time of 12 minutes, which is quicker than both MTA and BC Putty. Biodentine® was found to have a finer particle size when compared to MTA that helps to limit the porosity within the material (51). The material benefits from the lack of staining potential when compared to commonly used materials (44,52) which has made it a viable option for regenerative endodontics (53) and vital pulp therapy (54) where discoloration is a concern. The material has been shown to have similar patterns of inflammation when compared to MTA or BC Putty (55). Biodentine® is growing in its usage of direct and indirect pulp therapy due its similar outcomes compared to MTA (56) in clinical trials. The material shows a positive response to both pulpal and apical tissues and its help in dentin bridge formation (57). Septodont, the manufacturer of Biodentine®, also claims that the material should be used as a root end filling material suitable for surgery. After an investigation of the currently available research, there are limited cases or studies that show the use of Biodentine® for this purpose. One of the possible reasons is due to the lower radiodensity compared to either MTA or BC Putty (58,59). In fact, the material is touted as a “dentine replacement” and appears very similar to dentin in its radiopacity, which may make it less desirable for clinicians.

## BLOOD CONTAMINATION

Ideally during an apical surgery, a dry field is thought to be the best scenario. If that is not possible, there has been research showing difference in these previously mentioned materials. If blood contamination is a higher risk for a specific apical surgery, the use of orthograde filling of the canal space with MTA will not affect the apical seal so long as 3mm remains after resection (60). There is also no difference in orthograde placement of MTA or of freshly placed retrograde MTA after apical retropreparation (61). In the event of greater bleeding during a surgery, the use of white MTA has been shown to display greater microhardness when compared to gray MTA, thus being a better choice in material (62). In a blood contamination study, push-out bond strength of MTA and Biodentine® were compared, it was found that Biodentine® displayed no change with or without blood contamination (63). In a classical study, comparing the four retrofilling materials at the time (amalgam, IRM, Super EBA, MTA) and blood contamination, it was found that MTA proved to seal the root end preparation significantly better than the other materials (64). When comparing blood contamination in an in vitro leakage model, it was shown that Biodentine® and MTA are very comparable and that Biodentine® has no effect with blood present (65). In a more recent article, MTA and Biodentine® were compared and the surface and vertical dimensional changes were analyzed. The authors found that wet, dry, and blood conditions had a time dependent effect on the surfaces

of these materials. However, acidic conditions did not change the surface roughness or the level of these materials (66).

## SILVER NITRATE

Silver nitrate has had many uses since it's discovery. It has been used in the medical field as a cauterizing agent and is potent enough that it can be used in wart removal. Silver nitrate is also a radiopaque substance that shows up on conventional radiology films and has been shown to cause confusion if not properly diagnosed (67). In the field of dentistry, silver nitrate has been used as a tracing agent with micro-CT evaluation. It has been used to look at microleakage under dental restorations, to compare composite shrinkage (68), gap formation, bonding interface of orthodontic brackets (69), and others. This method allows for 3D reconstruction using micro-CT of the different materials due to the differences in their ability to attenuate the x-ray. The material has been shown to be more acidic in solution, which might raise questions about its use as a tracer for biological purposes. However, as seen in recent study, acidic conditions should not affect the surface characteristics of MTA or Biodentine® (66).

## MICRO-CT

Micro-computed tomography (micro-CT) has been used in endodontics for the last few decades. Because of the detailed images and accuracy, the technology is well suited for research in endodontics (70,71). Since its inception,

anatomical studies have been performed to give clinicians a more in-depth look at the complex design of each tooth type (72–74). Micro-CT studies are also used to study instrumentation of canals. One of the drawbacks to micro-CT imaging is the time and expense associated with the machine itself and processing the acquired images. However, the results can prove to be spectacular and display much more information than what is presented historically from anatomical studies using dye or sectioning methods (75). Micro-CT allows for precise measurements compared to macroscale views of teeth as in the past. Micro-CT has also been implemented for in vivo animal models to look at dentin formation after regenerative procedures (76) or vital pulp therapy (77).

The basic operating design of a micro-CT system is that an x-ray source releases energy toward the sample to be scanned, and the data is collected in an x-ray-to-electronic signal-converting imaging array. All machines have a platform where the sample is placed for scanning to occur. The difference comes in whether the sample and platform move or whether the x-ray source moves (78). Micro-CT imaging has been shown to be reliable for measurements, as well as a non-destructive way of evaluating tooth structures and tissues (70). This imaging modality converts the attenuation into greyscale values that can then be used to differentiate between various materials or tissue types. For the purposes of this study, the difference between Biodentine®, dentin, silver nitrate, blood, and voids were analyzed with micro-CT.

## SPECIFIC AIMS

1. To evaluate the sealing ability of Biodentine® in its use as a root end filling material.
2. To evaluate how the sealing ability of Biodentine® would be affected by contamination with blood.
3. To evaluate the use of micro-CT and silver nitrate as a method to examine sealing ability and the microleakage of different dental materials.

## HYPOTHESIS

The sealing ability of Biodentine® will be affected by blood contamination and allow silver nitrate to penetrate into and along the walls of the root end filled area. There will be an increase in the volume of voids found throughout the root end filled area.

## NULL HYPOTHESIS

The sealing ability of Biodentine® will not be affected by blood contamination and there will be no difference in silver nitrate penetration into and along the walls of the root end filled area. There will be no difference in the volume of voids found throughout the root end filled area.

# MATERIALS AND METHODS

## 1. Pilot Study

The use of silver nitrate as a tracer for micro-CT has been established (68,79). For the purposes of this pilot study, ProRoot White MTA™ (Dentsply Sirona Inc., York, PA, USA) and Endosequence® BC RRM-Fast Set Putty™ (BC Putty)(Brasseler, Savannah, GA, USA) were compared to determine the extent of the silver nitrate penetration and to determine if there was a difference in the radiopacity of the silver nitrate and the radiopacifiers found within the materials. Since these two materials are the most widely used root end filling materials, they were selected for this study.

Four previously extracted human teeth were used for the pilot study. The inclusion criteria for the teeth were that they be single rooted, permanent teeth with a single apical foramen. Excluded teeth were multirooted teeth, teeth with open apices, teeth with resorption, previously treated teeth, and teeth with visible cracks. The use of human teeth was approved by the University of Minnesota Institutional Review Board as an exemption of not human research. The teeth were stored in 10% formalin and the teeth were cleaned by removing residual soft tissues and hard deposits attached to the root surfaces. The teeth were decoronated. The teeth were prepared by cleaning and shaping of the canal using the ProTaper Gold file system (Dentsply Sirona Inc., York, PA, USA), enlarging to the F3 file shape and size. Sodium hypochlorite (6%) was used as

an irrigant throughout the shaping process to eliminate residual pulp tissue left in the canal and to act as a lubricant during the instrumentation process. The smear layer was removed with a 17% EDTA and sodium hypochlorite rinse before obturation (80). The teeth were then obturated using the warm vertical compaction technique with gutta-percha and AH Plus sealer. The teeth were then apically resected 3mm using a multipurpose bur. Two of the samples were resected at a 45° bevel angle and two resected at a 0° bevel angle as possible. The apical gutta-percha was removed using an ultrasonic tip up to 3mm. The area was rinsed and dried using a combination of paper points and air from the Stropko syringe tip. Two samples of MTA and BC Putty were placed into each of the two resection bevel angles.

*Figure 1*. Top image shows the four prepared samples for the pilot study, 2 containing MTA and 2 containing BC Putty. Lower left image is silver nitrate in its white crystal form. Lower right image shows the resected root end after silver nitrate soak.

To prepare for the scanning process, each tooth sample was coated with nail polish, leaving only the apical resected area exposed to the solution. The samples were numbered using a dot system of one to four dots painted on the sample with a different color nail polish. The four samples were soaked in a 10% w/w silver nitrate solution (Chem-Impex Int'l Inc., Wood Dale, IL, USA) overnight to ensure proper time for penetration. To create the solution, silver nitrate crystals were dissolved in distilled water. After rinsing in distilled water to remove any excess silver nitrate, the teeth were dried and ready for mounting.

In order to maximize both scan time and space, a method to stack the samples vertically was developed. The samples could not be in contact with another one, nothing radiopaque could be used, and all while keeping the dimension as small as possible vertically. For the micro-CT to be most accurate, the samples must be rigid enough to not move throughout the ~20-minute scanning time and be mostly free standing. A previous study conducted at the University of Minnesota (79) used a type of super glue, Krazy Glue™ (Elmer's Products, Columbus, OH, USA) to adhere tooth furcation samples to one another after being coated with nail polish, similar to this study. In order to create separation between the samples, a Microbrush® (Microbrush International, Grafton, WI, USA) that is commonly used in dental procedures was used as a backbone to which the samples would be adhered. The Microbrush® was rigid enough to hold the samples but also adhered well with the chosen super glue for this study, Loctite® Super Glue Liquid (Henkel Corporation, Rocky Hill, CT, USA). In order to have the sample free-standing, the Microbrush® and four

adhered samples was placed inside a sterile plastic container used for packaging a 3mL tuberculin syringe (Covidien/Medtronic, Minneapolis, MN, USA). With the syringe removed, these plastic containers are hollow and large enough to hold the samples on the Microbrush®. The base of the container is wider than the rest of the container which made it balance and be more free standing. The tops of the plastic containers had to be removed to allow space for the samples to fit inside. A model trimmer was used to remove the top portion of the containers. Loctite® Super Glue Liquid was used to adhere the Microbrush® to the plastic container for the scanning. Once the Microbrush® was attached to the plastic container, it was ready to be scanned.

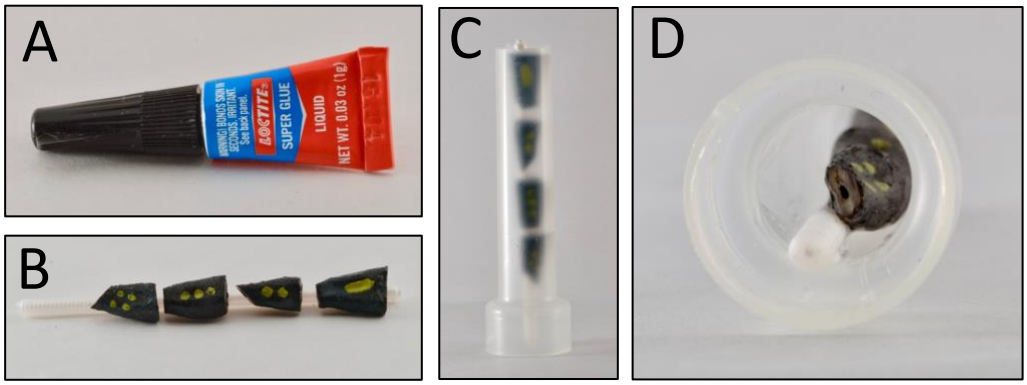


*Figure 2.* Loctite® Super Glue Liquid (A) used to adhere samples to Microbrush® (B). Samples adhered vertically (C) and inside the hollow syringe container (D).

The micro-CT unit (H225 X-teck XT, Nikon Metrology Inc., Brighton, MI, USA) is located on the 16$^{th}$ floor of Moos Tower. The scanning parameters were set at 105kV, 75 μA, 708ms of exposure, 720 projections and 2 frames per

projection. Initially, the samples were larger vertically and so two teeth were scanned at a time to compare the radiopacity of the materials. In order to ensure no movement in the scan, modeling putty was placed on the scanning plate and the samples in the plastic component were pushed into the center of the putty for more stability. Scanning was completed and the scans were then uploaded in the software application CT Pro 3D software (Nikon Metrology Inc., Brighton, MI, USA) for processing and 3D conversion. This 3D reconstruction was then uploaded into VGStudio Max 3.1 software (Volume Graphics GmbH, Heidelberg, Germany) to interpret the scans and reconstruction to verify the radiopacity of the materials before moving on in the study.

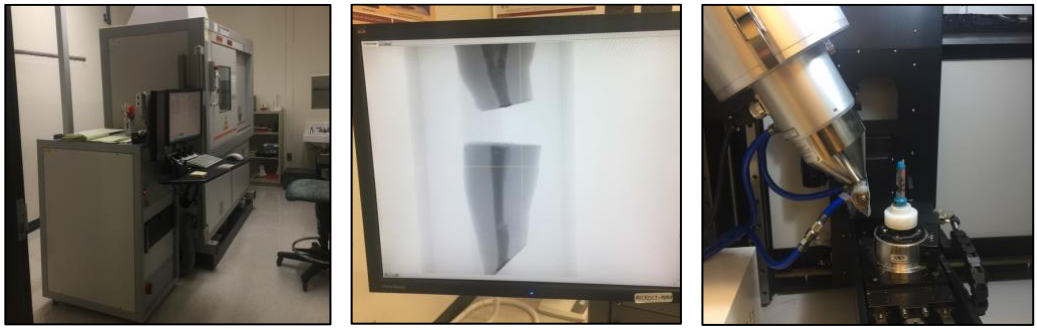

*Figure 3.* Micro-CT unit located on the 16th floor of the University of Minnesota School of Dentistry. Right image shows the sample being scanned as part of the pilot study.

After examination of the BC putty and MTA samples in the software, it was clear that the radiopacity of the silver nitrate was too similar to that of the two materials. These materials have radiopaque particles speckled throughout the

material which would make it difficult to analyze compared to the silver nitrate particles. Due to this complication, a decision was made to test Biodentine®, MTA and BC Putty without any tracer present to verify which of the three materials would be more suited for this experiment with the silver nitrate tracer.

In order to test this hypothesis, three more samples were prepared as described above. MTA, BC putty and Biodentine® were each placed in their respective samples and coated with nail polish. Different secondary nail polish colors were used to label each material: white for Biodentine®, red for MTA and

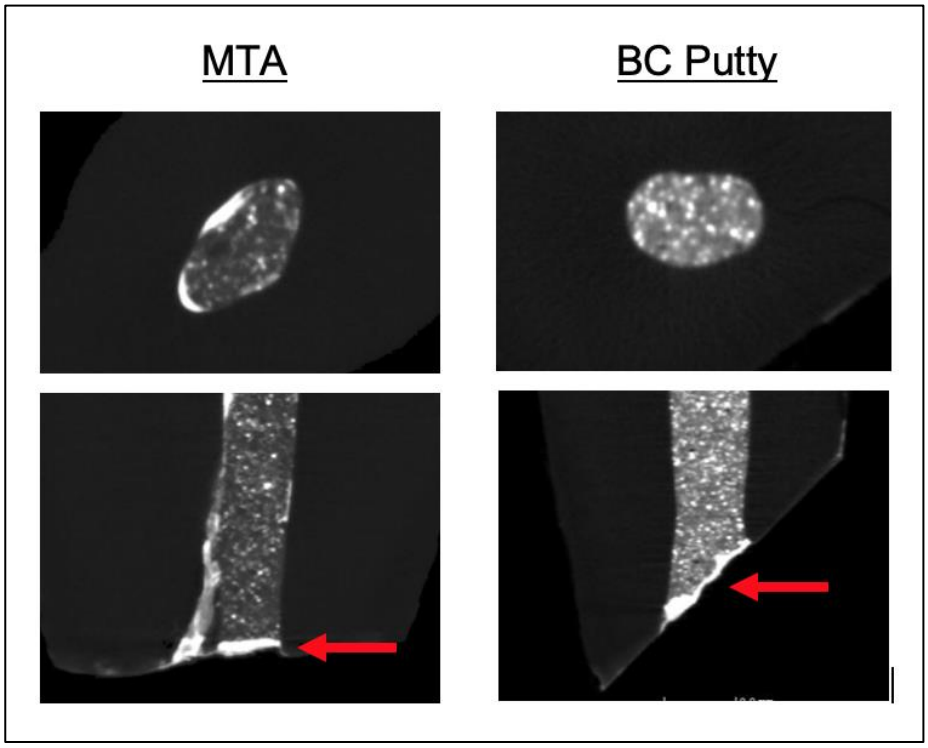


*Figure 4.* Axial and transverse slices of MTA (left side) and BC Putty (right side) showing radiopacifier particles throughout the filling material. Red arrows show silver nitrate penetration along resected portion of sample teeth.

blue for BC Putty. The samples were adhered to the microbrush using Loctite® Super Glue Liquid and subsequently adhered to the plastic syringe container. In order to improve the stability of the Microbrush® to the plastic container, PVS impression material (Aquasil Ultra LV, Dentsply Sirona Inc., York, PA, USA) was injected at the top and bottom of the plastic container, completely surrounding the Microbrush® to maintain it within the center of the container. These three samples were scanned using the micro-CT scanner but without any silver nitrate tracer. The three materials were compared (see figure 5) and it was determined that Biodentine® is better suited as the retrofilling material for this study design.

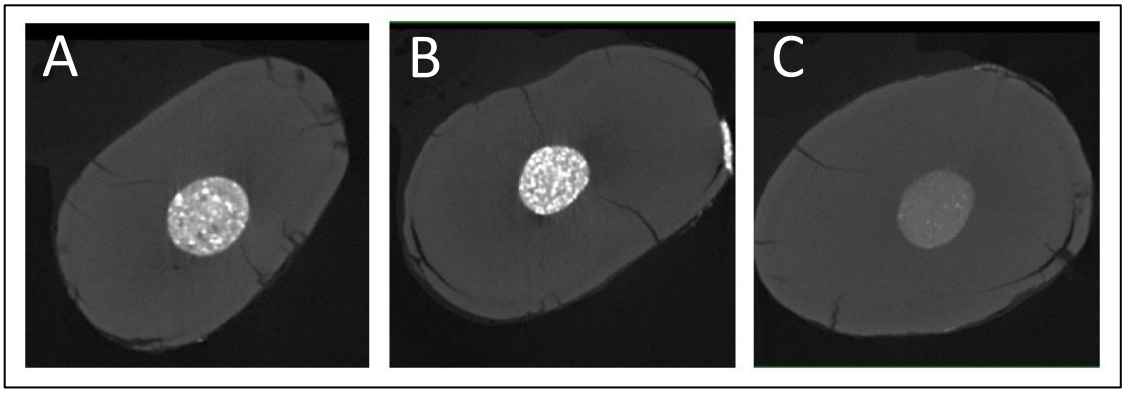


*Figure 5.* Axial slices showing the difference in radiopacity between the three retrofilling materials: MTA (A), BC Putty (B), and Biodentine® (C)

## 2. Sample Preparation

Thirty single rooted teeth were selected as sample teeth. The teeth were extracted from the Oral Surgery Division of the University of Minnesota School of

Dentistry during the fall of 2018 with approval by Dr. Nadeau of the Oral Surgery Department. Teeth were included as described previously in the pilot study section. Sample teeth were prepared and obturated as described above. The samples were coated with nail polish, resected 3mm apically with a 0° bevel angle.

A system for labeling the sample teeth was developed by using various nail polish colors. After samples were randomly selected, they received their grouping of either test group, contamination group, positive control, or negative control. Each tooth was primarily coated with black nail polish and then with another color nail polish to differentiate the samples. To distinguish between sample groups, a secondary color (blue) was added to the trimmed coronal portion of the blood contaminated samples. A dot color of a different nail polish color was placed on top of the black nail polish in one, two or three dots. See *Table 1* for the labeling system created to organize the samples.

| Sample | Primary Color | Secondary Color | Dot Color | # of Dots |
|---|---|---|---|---|
| 1 | Black | | Red | 1 |
| 2 | Black | | Red | 2 |
| 3 | Black | | Red | 3 |
| 4 | Black | | White | 1 |
| 5 | Black | | White | 2 |
| 6 | Black | | White | 3 |
| 7 | Black | | Yellow | 1 |
| 8 | Black | | Yellow | 2 |
| 9 | Black | | Yellow | 3 |
| 10 | Black | | Green | 1 |
| 11 | Black | | Green | 2 |
| 12 | Black | | Green | 3 |
| 13 | Black | Blue | Red | 1 |
| 14 | Black | Blue | Red | 2 |
| 15 | Black | Blue | Red | 3 |
| 16 | Black | Blue | White | 1 |
| 17 | Black | Blue | White | 2 |
| 18 | Black | Blue | White | 3 |
| 19 | Black | Blue | Yellow | 1 |
| 20 | Black | Blue | Yellow | 2 |
| 21 | Black | Blue | Yellow | 3 |
| 22 | Black | Blue | Green | 1 |
| 23 | Black | Blue | Green | 2 |
| 24 | Black | Blue | Green | 3 |
| 25 | Black | | Blue | 1 |
| 26 | Black | | Blue | 2 |
| 27 | Black | | Blue | 3 |
| 28 | Black | 1 blue dot | White | 1 |
| 29 | Black | 1 blue dot | White | 2 |
| 30 | Black | 1 blue dot | White | 3 |

*Table 1*. Labeling system developed for labeling each of the 30 samples. Different colors denote nail polish colors applied to the tooth which served to block out unwanted silver nitrate penetration.

With the samples labeled, each was apically prepared using an ultrasonic root end preparation tip. This process was completed under the DOM to ensure consistency and that all gutta-percha was removed at least 3mm. Digital

radiographs were taken of each sample using RVG 6200 sensors (Carestream Dental LLC, Atlanta, GA, USA) to ensure that there were no gutta-percha or sealer remnants in the apically prepared canal space. If there were any remnants left on the walls, attempts were made to remove the remnants before moving on to the retrofilling of the samples.

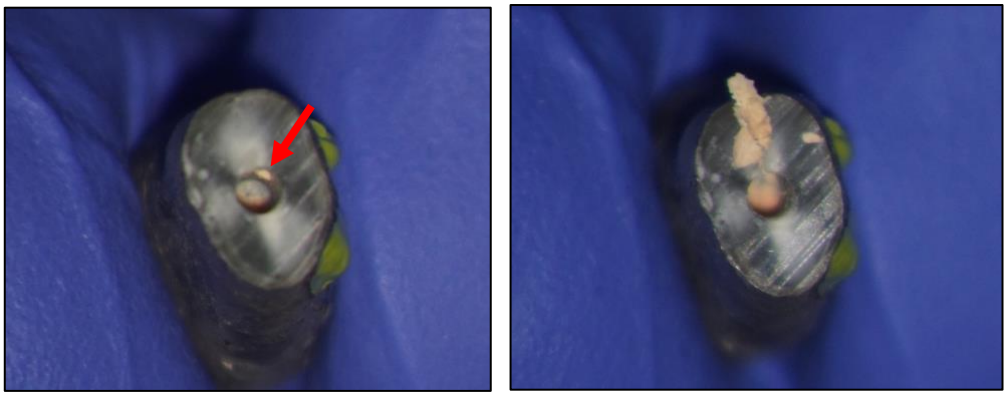

*Figure 6*. Example of retropreparation completed under the dental operating microscope. Left image shows gutta-percha remaining on canal wall (red arrow). Right image shows removed gutta-percha.

Freshly mixed Biodentine® was placed into the root end preparation using a 1/2 Hollenback Carver instrument (Hu-Friedy Mfg. Co., LLC, Chicago, IL, USA) until flush with the resected apex. Digital radiographs were taken to verify adequate filling of the area and to ensure that there were no voids present. If a void was noted, more material was added, and another radiograph was taken (see figure 7). Due to the quantity dispensed in each Biodentine® capsule, several samples were filled with each mixed capsule of material. When the

consistency of the Biodentine® became too dry, a new batch was mixed and used to complete the remaining samples. All mixing was completed following manufacturer's instructions and specifications. Once all samples were completed, each sample was immediately placed in an Eppendorf tube containing 1.5mL of sterile phosphor buffered saline (PBS) to simulate the oral fluids and a moist environment for which the Biodentine® would fully harden.

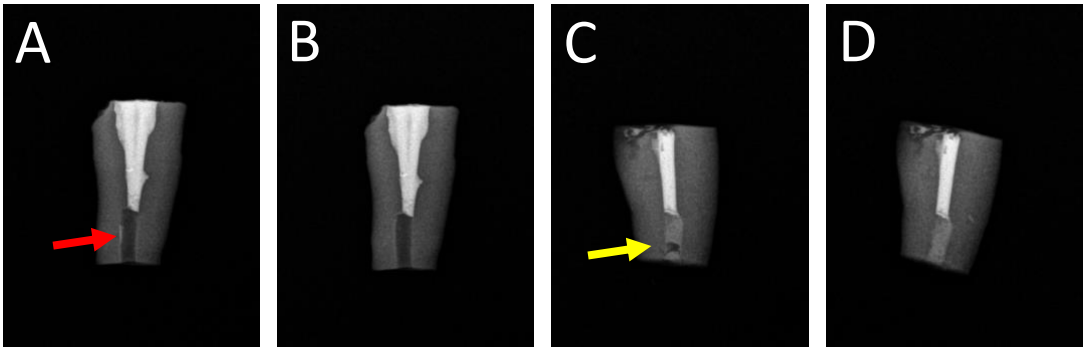


*Figure 7*. Radiograph shows retropreparation with remaining radiopaque material (red arrow) on dentin wall (A). Radiograph after removal of radiopacity from retropreparation (B). Radiograph with void (yellow arrow) present in Biodentine® retrofilling (C) and further condensing of material to remove void (D).

For the blood contaminated samples, whole, fresh blood was acquired from a healthy consented volunteer member of the research group (CD). With the help of a trained phlebotomist, 3ml of fresh blood was drawn and a portion was placed into a sterile 1ml Monoject™ tuberculin syringe (Covidien/Medtronic, Minneapolis, MN, USA). Twelve samples were root end prepared as above and rinsed and dried. One drop of the acquired blood was placed into each root end

preparation. The blood was left in place for 1 minute and then the retropreparations were air dried using a Stropko adapter for the air/water syringe. This left blood on the dentinal walls of the retropreparation area, even up to the gutta-percha remaining in the samples. Fresh batches of Biodentine® were prepared as above, the root end preparations were filled, and a radiograph taken. Some of the samples had residual blood that was incorporated into the retrofilling area during the compaction of the Biodentine® into the root end. The Biodentine® was filled to the apex and then the root end was cleaned with a moistened sterile cotton pellet to ensure a clean surface. If any voids were noted on the digital radiograph, more compaction was completed to ensure no voids (see example in Figure 7). The blood contaminated samples were then immediately placed into sterile PBS in their labeled Eppendorf tube.

Three positive controls and three negative controls were used for this experiment. The positive controls were prepared as previously described, to the point of root end preparation. The root end preparations were left unfilled thus allowing full exposure to the silver nitrate solution. The negative controls were filled with Biodentine® after retropreparation and then covered with black nail polish to seal the root end from exposure to the silver nitrate solution. The control groups were subsequently placed in PBS solution in their labeled Eppendorf tubes.

All of the prepared sample groups, including the control groups were left in the PBS containing Eppendorf tubes for one week to allow for proper setting of

the Biodentine® material. Prior to silver nitrate exposure, each of the samples were trimmed so that each sample was approximately 6mm in length.

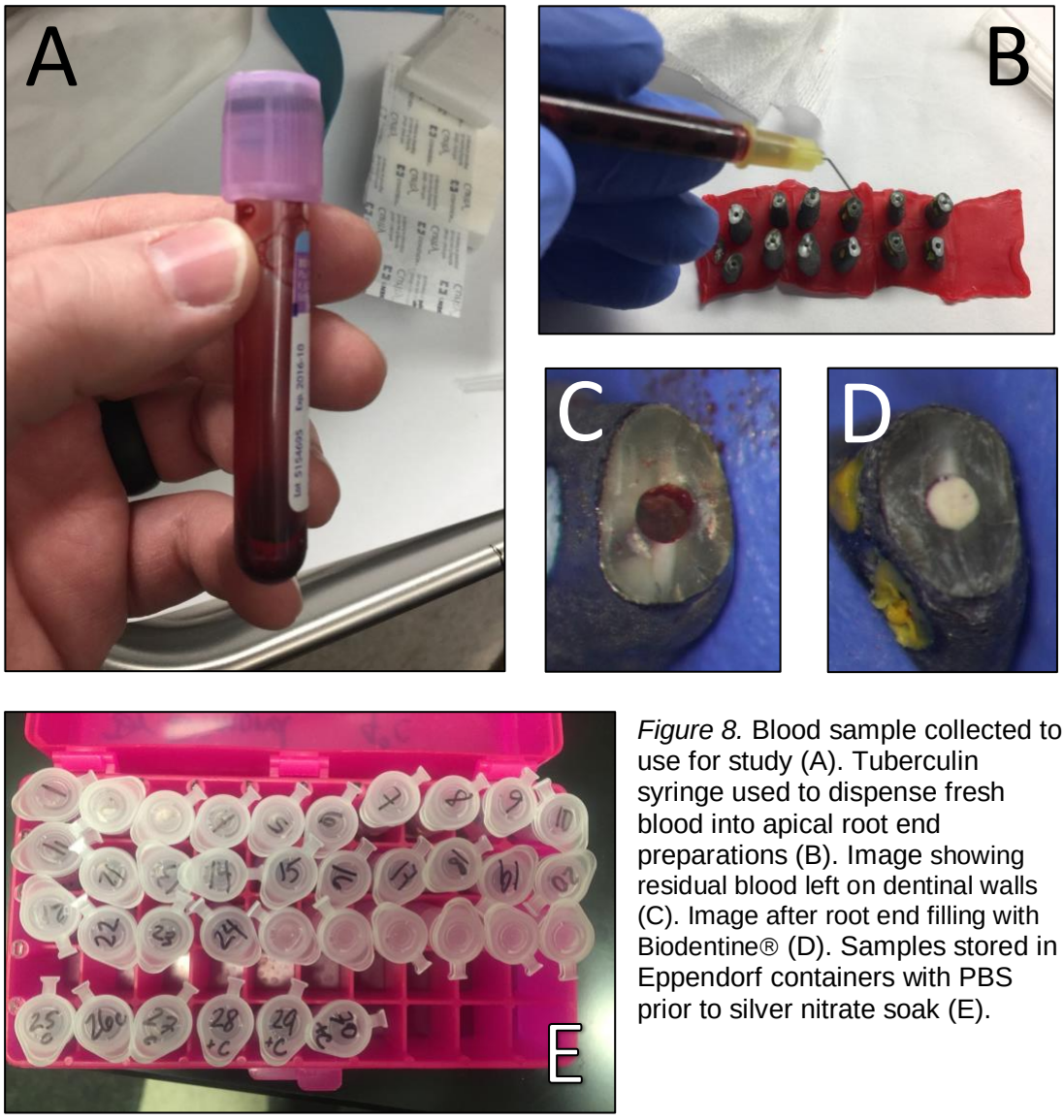


*Figure 8.* Blood sample collected to use for study (A). Tuberculin syringe used to dispense fresh blood into apical root end preparations (B). Image showing residual blood left on dentinal walls (C). Image after root end filling with Biodentine® (D). Samples stored in Eppendorf containers with PBS prior to silver nitrate soak (E).

### 3. Silver Nitrate Exposure and Scanning

Based on prior research by Brown (2017), it was decided a 25% w/w solution of silver nitrate would be used in this study. The solution was made by dissolving the crystal form of silver nitrate (10g) in distilled water (30g). The solution was mixed until no silver nitrate precipitate remained and all was dissolved into the distilled water. The estimated pH of the silver nitrate solution was approximately 3.0. The solution was placed in a 100ml container and the sample teeth were added to the solution. The samples remained in the silver nitrate overnight to ensure proper exposure of the samples. After 24 hours, the samples were rinsed in distilled water to remove excess silver nitrate and then placed into a distilled water container overnight. The next day, the samples were removed from the distilled water container. The samples were dried, and a digital radiograph was taken of each sample.

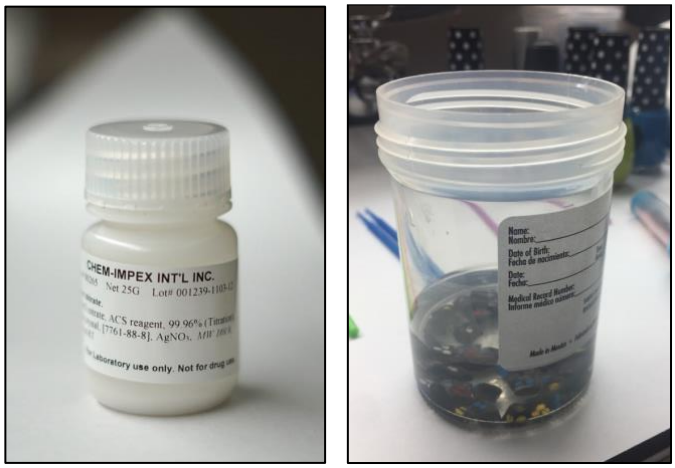


*Figure 9.* Left image is of silver nitrate crystals used to create the solution. Right image is showing the full immersion of the samples in the dissolved silver nitrate solution. Samples were left over night to allow for complete penetration of each sample.

The samples were sorted into their corresponding groups based on the color-coding system described earlier. Each group of three was then adhered to a Microbrush® of corresponding color to match the dot color on the sample group (i.e. yellow dot sample group mounted on yellow Microbrush®). Each Microbrush® was then adhered to the previously prepared plastic syringe containers. Care was taken to center the samples within the plastic container to allow for a centered scan of the sample with the micro-CT unit. PVS impression material was placed at the ends of each container to stabilize the Microbrush® and then the containers were labeled on the outside. In all, 10 sample containers were constructed to hold the 30 samples and controls (see *Figure 10*).

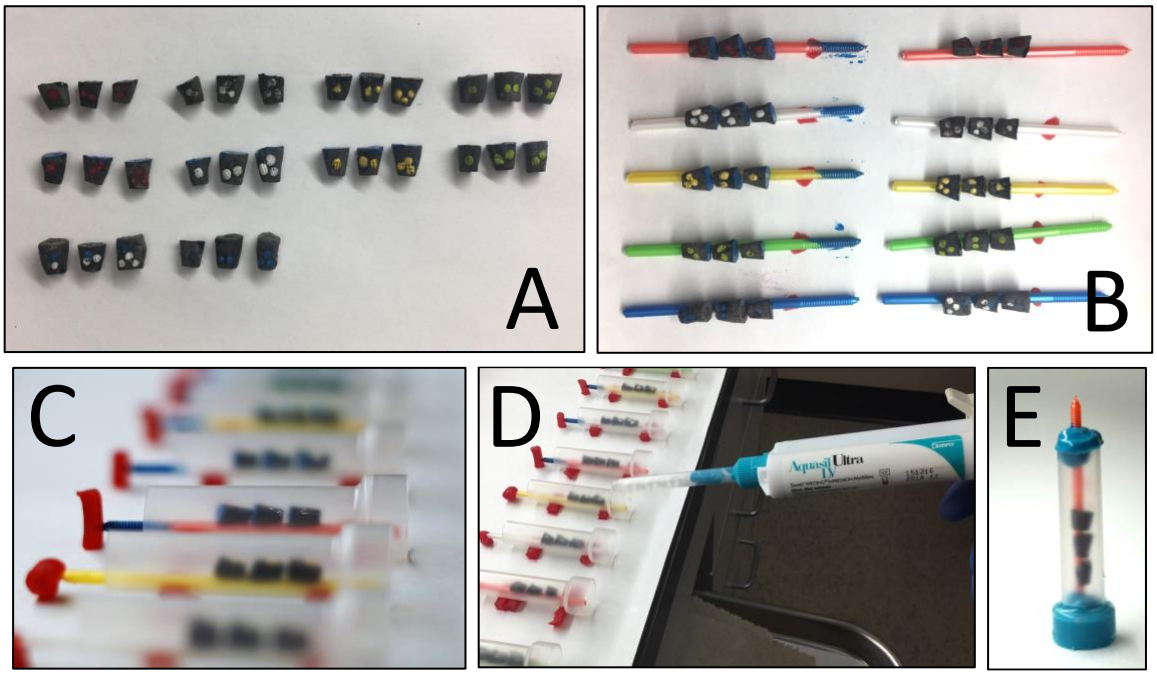


*Figure 10.* Process of sample preparation: (A) Dried and labeled samples, (B) Samples adhered to corresponding colored Microbrush® using Loctite® Super Glue Liquid and then adhered into plastic syringe containers (B, C), PVS material placed at top and bottom of containers to add stability (D), completed sample (E).

Scanning of the samples was performed using a micro-CT unit located on the 16th floor of Moos Tower at the University of Minnesota School of Dentistry. The scanning parameters used were 105kV, 75μA, 708ms of exposure, 720 projections and 2 frames per projection. The total scanning time was approximately 20 minutes for each plastic container of samples.

## 4. Image Processing

Three-dimensional reconstructions of each micro-CT volume was completed using CT Pro 3D (Nikon metrology, Inc., Brighton, MI, USA). Each sample was checked at two points to verify the center of rotation and that there was no movement of the sample during the scanning time (see *Figure 11*).

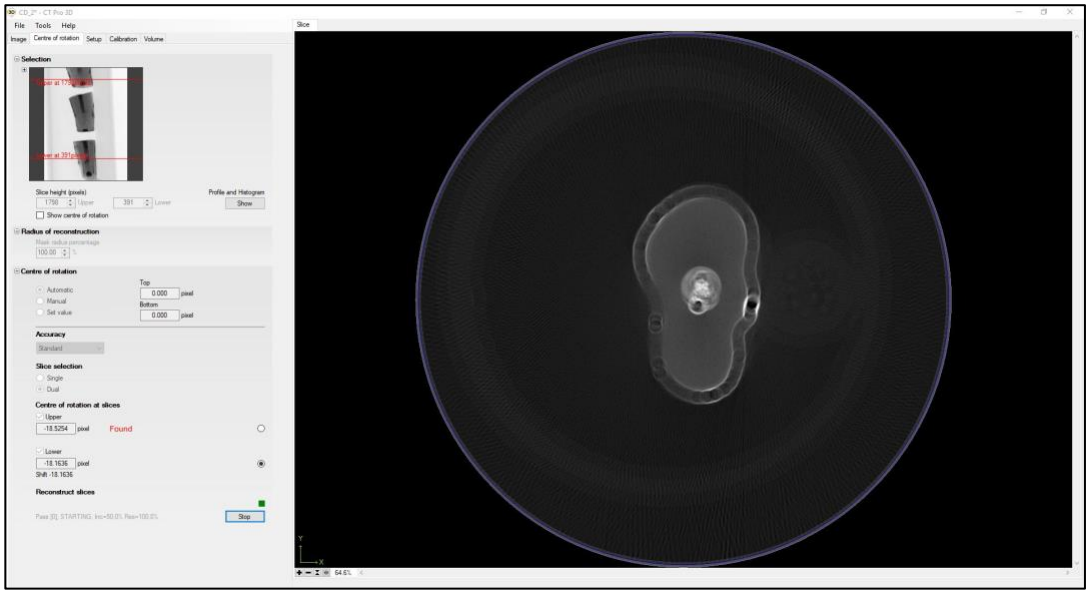


*Figure 11*. CT Pro 3D software used to ensure that there was no movement of each sample during the scanning process.

None of the samples required rescanning as they all had no movement during scanning time. Once no movement was confirmed, the scan was roughly trimmed to only include the area of interest of the scan and to cut down on storage space. The trimmed data was exported to have visualization and 3D rendering completed using VGStudio MAX 3.1 software (Volume Graphics GmbH, Heidelberg, Germany). Using this software, the scans were divided into individual samples and exported into image stacks of .bmp files of the axial slices of the samples. Due to the mounting procedure of the samples, the .bmp files were imported into another program, CT-Analyzer (Bruker microCT, Belgium) to further trim the samples to only include the desired data for each individual sample. DataViewer software (Bruker microCT, Belgium) was used to vertically correct the samples in both the coronal and sagittal planes. The aligned sample data was exported in transaxial slices to be reopened in the CT-Analyzer software again for data collection. Aligning the samples to be parallel to the resected root surface allowed for a standardization in the data collection process (see *Figure 12B*).

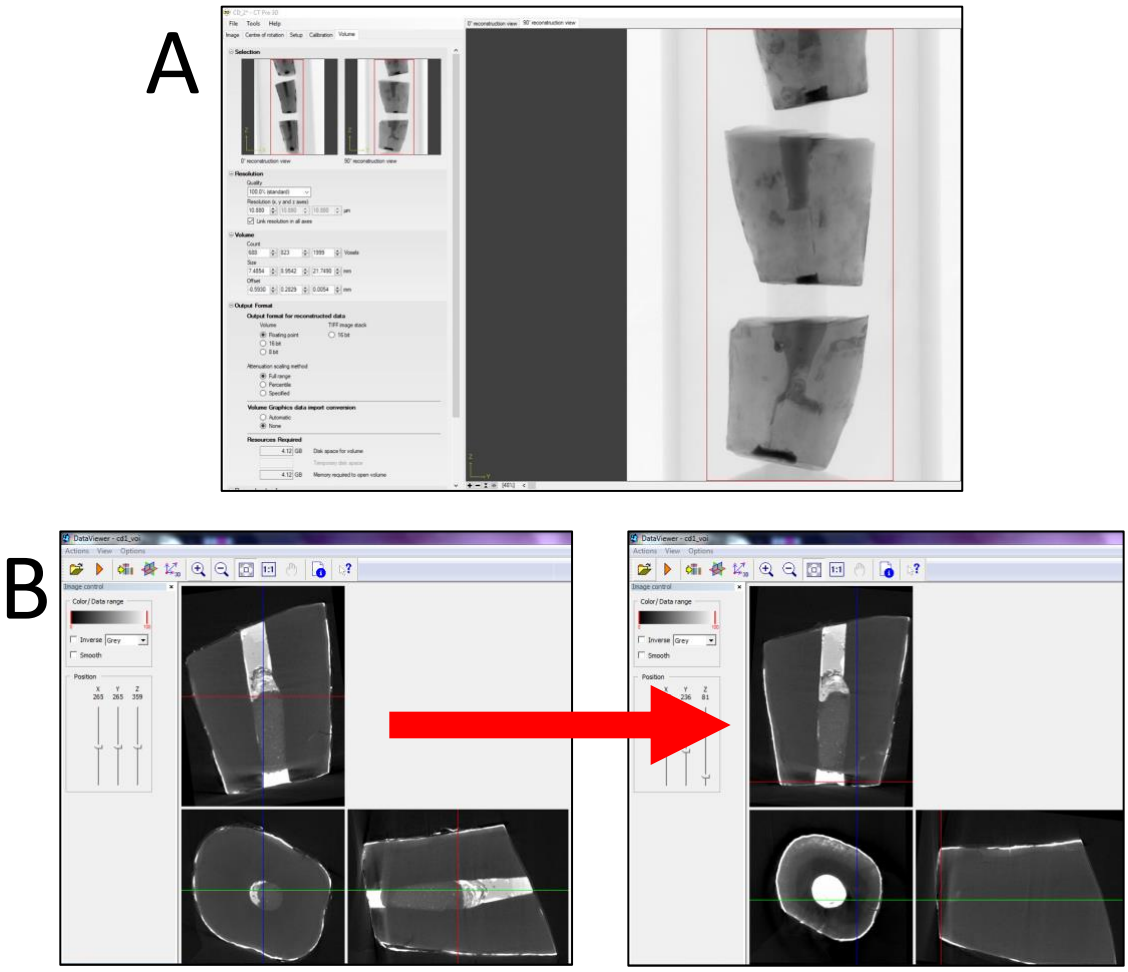


*Figure 12*. Example image of trimming of the scanned data to include only the area of interest around the sample. Red rectangular outline shows the trimmed area (A). Aligning of the sample using DataViewer software to ensure accurate measurement from the root end surface (B).

## 5. Regions of Interest and Data Collection

A single examiner (CD) was trained in the use of the CT-Analyzer software and carried out all the data collection and interpretation. For each sample, a Region of Interest (ROI) was created for the silver nitrate, Biodentine®, and voids in the sample. Each ROI required tracing the root end preparation of the sample as closely as possible to not include the surrounding

dentin. Apically, the ROI was started when a clear distinction between silver nitrate and scatter was visualized.

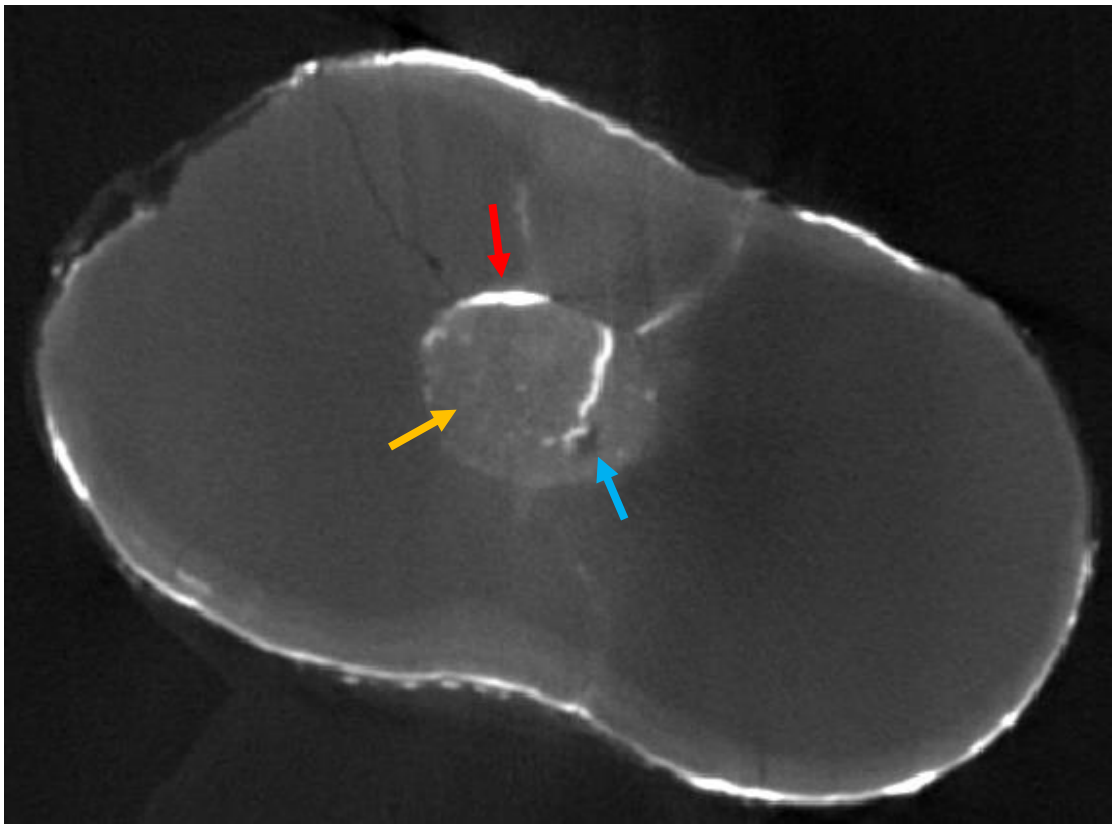

*Figure 13*. Example of a single axial slice that shows the outline of the tooth and the differences in density within the scanned area. Silver nitrate (red arrow), Biodentine® (yellow arrow), and a void (blue arrow) is seen in this slice.

At the junction of the Biodentine® and gutta-percha, the ROI started when a majority of the sample slice was Biodentine®. If there was sealer or gutta-percha remnants remaining in the canal, they could be excluded from the ROI and from the total calculations. Also, any cracks in the samples that filled with silver nitrate

were not included in the ROI. The radiographs that were taken during sample preparation where referenced to ensure that there were no remnants that needed to be cropped out of the ROI (see *Figure 14*). Each ROI required several tracings as you progressed up the sample slices from apex to the gutta-percha obturation. This proved to be the most tedious part of the data collection process due to the attention to detail required for each tracing. Once all the ROIs were completed for the 24 samples, each measured component (silver nitrate, Biodentine®, voids) could be excluded or included into the data set by adjusting the desired range from a binary mask scale. This scale ranges from 0-255, with the whitest pixels (silver nitrate) typically falling in the range of 150-255 and the darkest pixels (voids) in the lower end of the scale. The Biodentine® and voids are calculated together to be the overall root end preparation volume and typically fell in the range of 25-150 on the scale. The voids were then calculated alone and fell in the 25-60 range on the scale. The Biodentine® volume was the difference between the overall retropreparation volume and the void volume.

All the calculated volumes were entered into an Excel (Microsoft 2016, Redmond, WA, USA) spreadsheet. The values were adjusted due to the conversion of voxel size in the scan, which was set at 0.01125mm. This data was used to perform the statistical analysis.

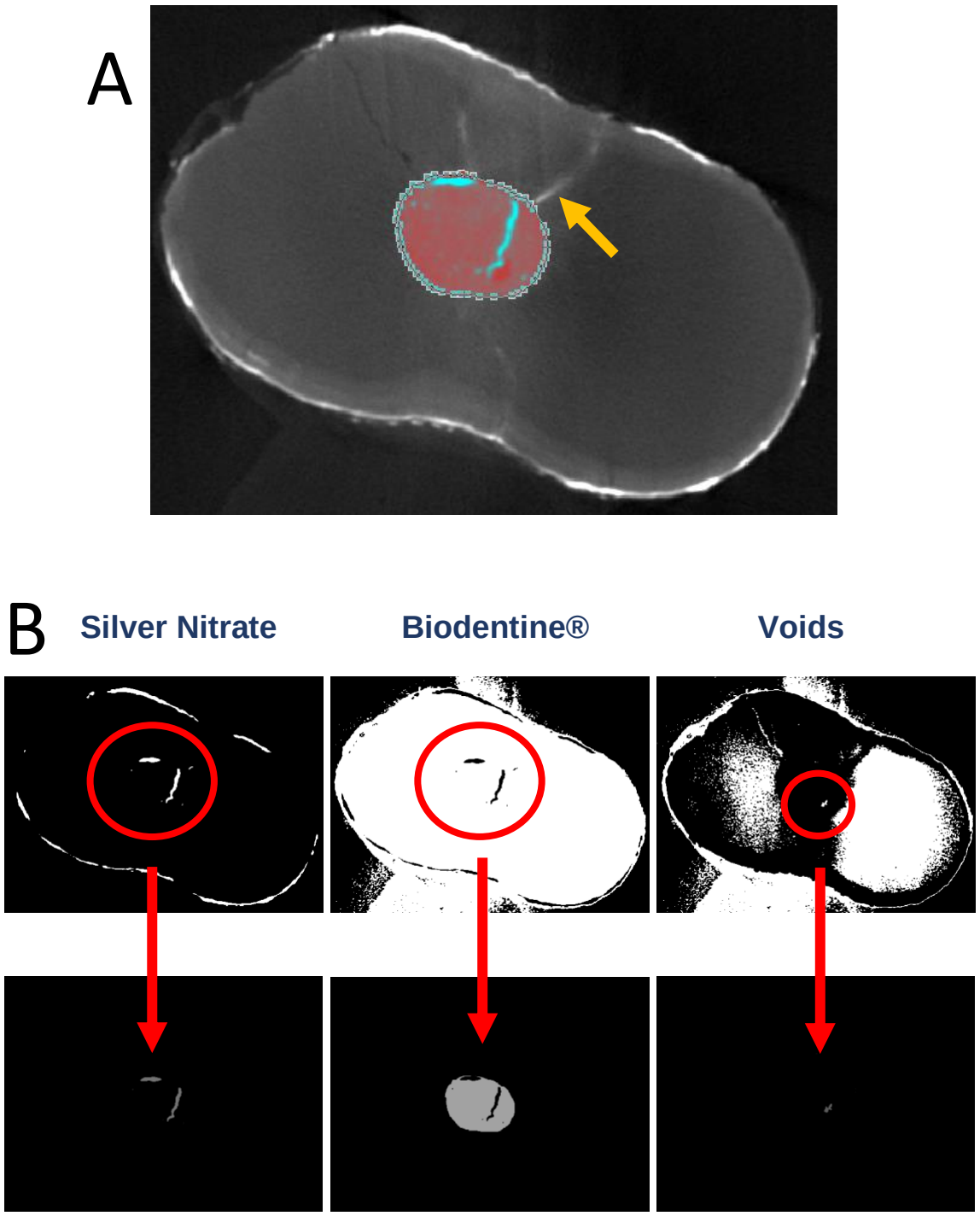


*Figure 14.* Example of detailed Region of Interest (ROI) traced on the axial slice of a sample, yellow arrow indicates a crack filled with silver nitrate that was not included as the ROI (A). Lower images show how each element (silver nitrate, Biodentine®, voids) were differentiated by using a Binary mask in the CT-Analyzer software to extract the volume of each component. The data only included that which was found within the ROI and nothing outside of the traced area (B).

Three-dimensional models were generated for each of the sample groups for demonstration purposes (*see Figure 16*). This was accomplished by using the CT-Volume software (Bruker microCT, Belgium) once all the ROIs were completed for each component. Positive and negative controls yielded the expected results: no silver nitrate penetration for the negative control, and complete penetration for the positive control (see *Figure 15*).

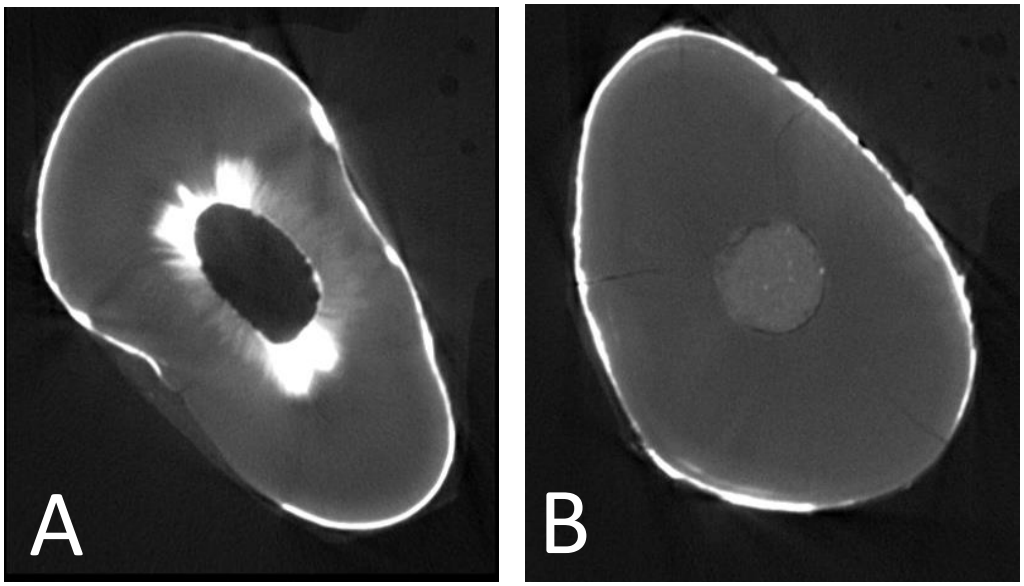


*Figure 15.* Positive and Negative Controls. Example of positive control showing silver nitrate penetration throughout the sample (A). Negative control without any penetration of silver nitrate tracer (B).

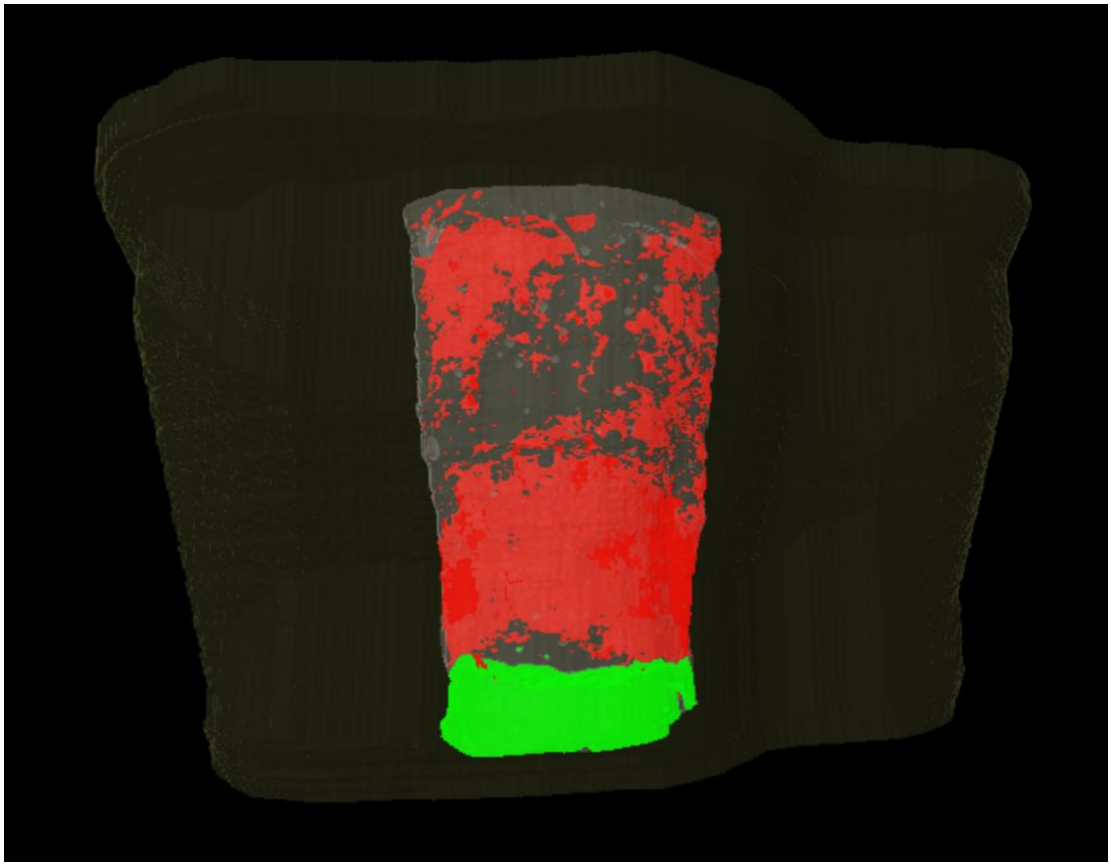

*Figure 16*. 3D model of a blood contaminated sample. Red represents voids in the root end filling material, green represents silver nitrate penetration into material, gray represents Biodentine®, and light yellow is dentin.

## 6. Statistical Analysis

For each component (silver nitrate, Biodentine®, voids), an object volume was calculated using the CT-Analyzer software. An overall volume of the root end preparation was calculated to compare each component in order to get a percentage. The depth of the root end preparation and depth of silver nitrate penetration were calculated according to the voxel size and the number of slices included for each sample. The data were entered into an Excel spreadsheet and

statistical analysis was completed using GraphPad Prism Version 8.1.2 statistical software (GraphPad Software, San Diego, CA, USA). D'Agostino-Pearson and Shapiro-Wilk normality tests were run on each dataset to confirm or deny Gaussian distribution of the data. If the data passed these normality tests, an unpaired t test was used to analyze the data. If the data failed the normality tests, a Mann-Whitney test was conducted. The level of significance was set at $p<0.05$. No samples were excluded from the study or dataset.

## Silver Nitrate Percentage of Root End Filling Volume

The percentage of silver nitrate compared to the overall volume of the root end filled area is given in *Figure 17*. The individual sample values can be found in Appendix I. An unpaired t test was performed to analyze the data values. The uncontaminated sample group is statistically different ($p = 0.0149$) from the blood contaminated sample group. Mean value for the uncontaminated group is 14.98% ± 4.78%. Mean value for the blood contaminated group is 9.84% ± 4.74%.

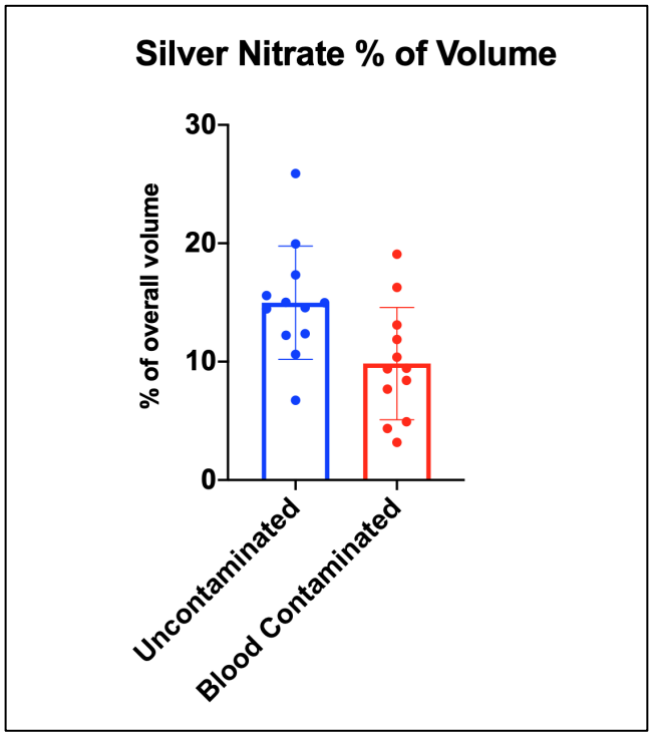


*Figure 17*. Silver Nitrate % of Volume scatter plot. Volume represents the total volume of root end filled area using Biodentine®. Values displayed in mean and standard deviation.

## Silver Nitrate Depth Penetration

The depth of silver nitrate penetration was compared between the two groups and is shown in *Figure 18*. The individual sample depth values can be found in Appendix I. An unpaired t test was performed to analyze the data values. There was no statistical difference between the two sample groups (p = 0.1097). Mean depth for the uncontaminated group is 0.73mm ± 0.16mm. Mean depth for the blood contaminated group is 0.63mm ± 0.13mm.

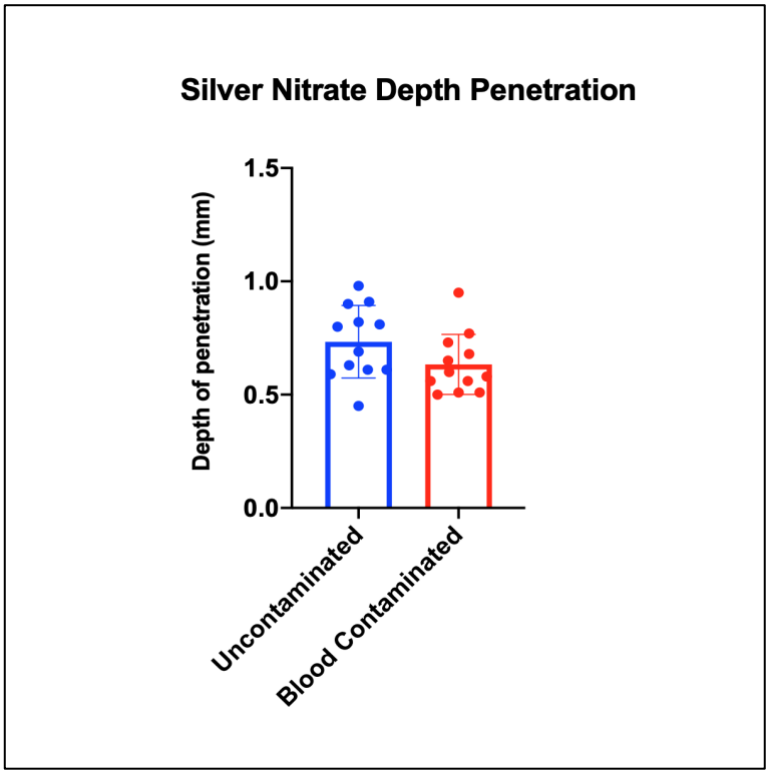


*Figure 18*. Depth of Silver Nitrate Penetration scatter plot. Values are displayed in mean with standard deviations.

## Silver Nitrate Penetration Depth as % of Root End Preparation Depth

The percentage of silver nitrate penetration depth was compared to the overall root end preparation between the two groups and is shown in *Figure 19*. Both values can be found in Appendix I. The Mann-Whitney test was performed to analyze the data values due to the data not displaying Gaussian distribution. There was no statistical difference between the two sample groups (p = 0.0859). Median value for the uncontaminated group is 24.36% with a range from 14.87% - 34.91%. Median value for the blood contaminated group is 20.81% with a range from 14.57% - 40.98%.

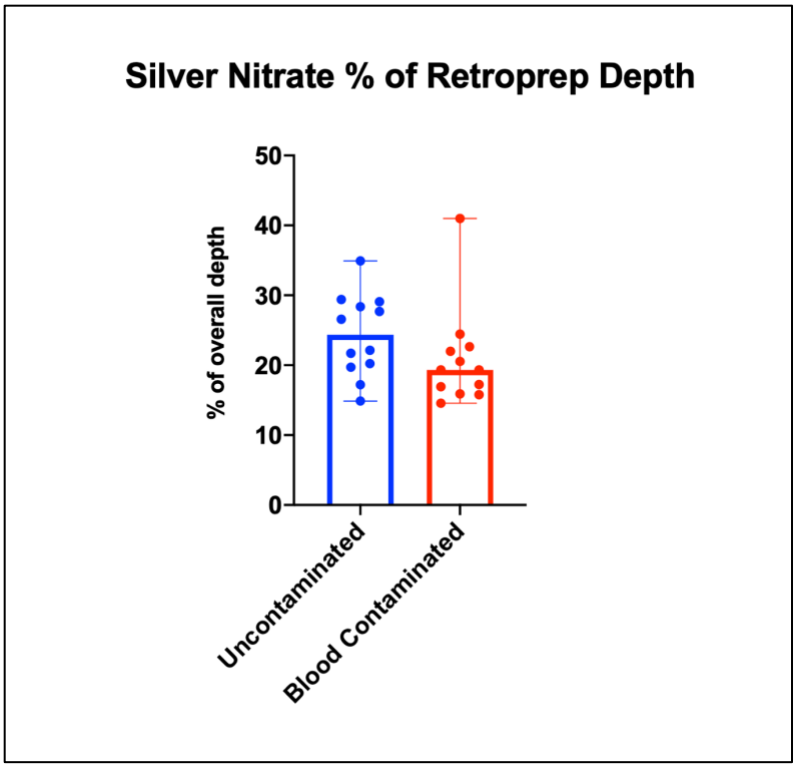


*Figure 19.* Silver Nitrate penetration depth as percentage of Root End Preparation Depth scatter plot. A Mann-Whitney test was performed. The values are presented using the median and range.

## Void Percentage of Total Volume

The void percentage of the total volume was compared between the two groups and is shown in *Figure 20.* The individual sample values can be found in Appendix I. An unpaired t test was performed to analyze the data values. There is a statistically significant difference between the two sample groups ($p < 0.0001$). Mean value for the uncontaminated group is 0.62% ± 0.69%. Mean value for the blood contaminated group is 4.75% ± 2.58%.

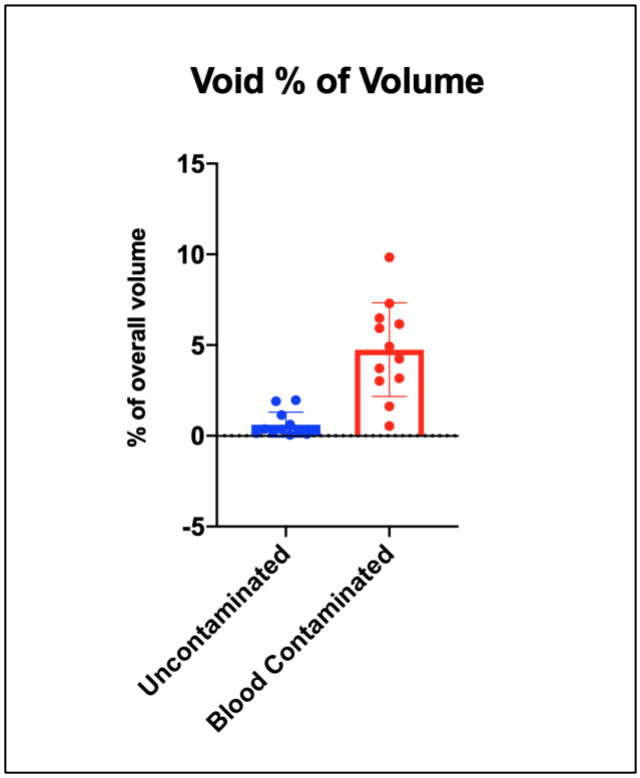


*Figure 20.* Void Percentage of Total Root End Filling Volume scatter plot. Values are displayed in mean with standard deviations.

# DISCUSSION

Success in surgical and non-surgical endodontics has consistently been tied to how we are able to seal the root canal space to prevent bacterial contamination. For surgery, these materials have evolved over time to produce modern bioceramic materials such as MTA, BC Putty and Biodentine®. Biodentine® is newest of these bioceramic materials used in endodontics and there remains a gap in research about this material, especially when used as a root end filling material. The product has primarily been used as a pulp capping agent or in regenerative endodontics due to its low staining ability but its lack of radiopacity on radiographs, which might deter clinicians from using it for surgery. The material exhibits a favorable working time, which is shorter than either MTA or BC Putty and has shown to create an adequate seal similar to these other materials.

In the current study, silver nitrate and micro-CT was used to evaluate the sealing ability of Biodentine®. This is the first documented use of this study design with Biodentine® based on the review of the literature. A previous study evaluated microleakage of Biodentine® and MTA using a glucose filtration method. This same study also evaluated blood contamination effect on microleakage and found that both materials performed equally well (65). Biodentine® and MTA have also been compared in a microleakage study using a silver nitrate dye solution, but the evaluation was completed using a stereomicroscope at 30x magnification. This study only quantified the depth of

penetration of the solution after the samples had been sectioned. Both Biodentine® and MTA were statistically significant compared to glass ionomer composite with this study model (81). Neither of these studies were able to quantify the volume of penetration or to three dimensionally evaluate the penetration and/or microleakage. Based on the silver nitrate penetration in the presented study, all the samples showed penetration less than 1mm into the material, which illustrates the importance of a 3mm retropreparation using ultrasonic tips and that a 1mm preparation may not be sufficient to seal the apex. Since all the samples were resected at a 0° bevel angle, there may be a need to evaluate the depth of penetration between different bevel angles in the future.

The results of this study showed that there was a statistical significance between the blood contamination group and the uncontaminated group when comparing silver nitrate percentage to the total volume of the root end preparation. The analysis showed that there was actually more silver nitrate penetration in the uncontaminated group, which was not the expected outcome. However, if the outlier samples were excluded from the data, or a smaller p-value selected, the results would suggest that there was no significant difference between the groups. This is an interesting finding because of the number of voids present with the blood contamination group that would traditionally be thought of as leading to more penetration. The setting reaction of Biodentine® may be the reason for there not being more penetration because Biodentine® sets when moisture is present. The Biodentine® could potentially use the blood as its source of moisture during the setting time. The samples were also placed

immediately into PBS after they were root end filled with Biodentine® and not allowed to fully set up before being exposed to more moisture. This PBS could have encouraged a fully set cure of the Biodentine® before it was placed in the silver nitrate. A study involving MTA and exposure to PBS showed expansion of the material, which may be why there was no penetration of the silver nitrate. The same study speculates that the proteins found within tissue fluids absorb into the materials and block porosities, which increases expansion in bioceramics (82).

All the samples showed some degree of voids in the placement of the retrofilling material. However, the blood contaminated group showed a much larger degree of voids compared to the uncontaminated group. This may be cause for concern as previous studies with MTA and BC Putty have shown that contamination lowers the physical properties of retrofilling materials. This is thought to be due to the voids not allowing a complete and cohesive filling material and the porosities/voids allow it to lose strength. There is very limited research about the physical properties of Biodentine®. However, in a recent study, MTA and Biodentine® were compared to see if there was any effect of blood and saliva contamination on the compressive strengths of the materials. The study found that the two materials are very comparable to one another in regards to compressive strength (83). Among the blood contamination samples, voids were typically evenly distributed throughout the root end preparation. An interesting observation was that voids in a majority of both sample types had voids at the interface of the initial obturation material and the Biodentine®.

Like most study designs, there were some limitations with this study. The first limitation is that this was an *in vitro* study and not a clinical trial or *in vivo* study. Great lengths were taken to follow current root canal preparation protocols during the cleaning, shaping, and obturation stages. Simulation of the root end surgery procedure is more difficult to recreate but the same instrumentation was used as a traditional surgery, and the samples were contaminated with a fresh blood sample and placed in PBS to simulate oral tissue fluids. By using extracted teeth, it was difficult to find samples that were perfectly similar as what may have been found in a simulated canal. The purpose of using real teeth was to allow the silver nitrate to penetrate the dentin tubules as bacteria do *in vivo*. Over the course of preparing the samples and scanning them, most of the teeth had developed some form of microcracks that could be visualized on the micro-CT images. The ROIs that were created for each sample required close attention to detail in order to not include any silver nitrate in the cracks as part of the counted data. Even with the cracks present, this did not affect the amount of silver nitrate penetration into the Biodentine® or along the walls of the root end preparation. One of the ways to improve this study would be to take scans of the samples with the micro-CT before and after, then use the subtraction method to distinguish between before and after treatment with the silver nitrate. This method has been used in prior studies (68) and may be an option going forward to investigate either MTA or BC Putty with this silver nitrate method. When comparing this study to other microleakage studies, it is important to look at the size of the tracers used and compare that to the size of bacteria. The average diameter of

bacteria is approximately 0.5-1.0 micrometers (84), the particle size of silver nitrate ions is 0.059nm in diameter. This size difference can greatly overestimate the amount of silver nitrate leakage that would be considered clinically relevant.

When looking at possible future studies, there is a lot of potential using this study design. Comparison studies using MTA, BC Putty and Biodentine® with silver nitrate could be accomplished with the subtraction method. The subtraction method should show promise because the radiopacity of materials is less of an issue if you have before and after micro-CT scans. Silver nitrate should continue to prove valuable when comparing restorative materials in endodontics as well. For example, testing various orifice barriers or build up materials for microleakage and dentin bonding. This would help to ensure that the coronal aspects of root canals maintain a tight seal, which is the goal of our endodontic treatment for preventing contamination of the canal space. This method would allow three-dimensional analysis of microleakage which would be an improvement from the previous dye methods used to evaluate coronal leakage. Different tooth types or anatomical variations could also be a future direction in using this method. An evaluation of isthmuses of molars, bevel angle variations, compaction of materials methods, and others.

# CONCLUSIONS

1. Blood contamination does not affect the sealing ability of Biodentine®, when it is used as a root end filling material.

2. The presence of blood in the root end preparation significantly increases the volume of voids present in the root end filling material

3. The combination of silver nitrate as a tracer and micro-CT to evaluate microleakage is a promising study design for future applications

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# APPENDIX I

| | Sample # | Total Retroprep Volume (µm^3) | Silver Nitrate Volume (µm^3) | Silver Nitrate Penetration Depth (mm) | Void Object Volume (µm^3) | Retroprep Depth (mm) | Biodentine volume (µm^3) | Silver Nitrate % of total vol | Void % of total volume | Biodentine % of total volume | Silver nitrate % of retroprep depth |
|---|---|---|---|---|---|---|---|---|---|---|---|
| uncontaminated | 1 | 361.25 | 52.25 | 0.82 | 1.28 | 2.83 | 307.71 | 14.46% | 0.35% | 85.18% | 29.08% |
| | 2 | 405.55 | 63.24 | 0.90 | 7.75 | 3.18 | 334.55 | 15.59% | 1.91% | 82.49% | 28.37% |
| | 3 | 455.69 | 66.33 | 0.69 | 0.24 | 3.17 | 389.12 | 14.56% | 0.05% | 85.39% | 21.71% |
| | 4 | 375.14 | 97.16 | 0.91 | 7.40 | 2.62 | 270.59 | 25.90% | 1.97% | 72.13% | 34.91% |
| | 5 | 433.80 | 53.06 | 0.61 | 0.43 | 3.54 | 380.31 | 12.23% | 0.10% | 87.67% | 17.20% |
| | 6 | 626.09 | 108.48 | 0.61 | 7.23 | 2.75 | 510.39 | 17.33% | 1.15% | 81.52% | 22.13% |
| | 7 | 460.18 | 31.02 | 0.80 | 1.00 | 3.01 | 428.17 | 6.74% | 0.22% | 93.04% | 26.59% |
| | 8 | 464.74 | 57.44 | 0.59 | 0.66 | 2.90 | 406.63 | 12.36% | 0.14% | 87.50% | 20.23% |
| | 9 | 491.19 | 73.63 | 0.98 | 1.82 | 3.34 | 415.74 | 14.99% | 0.37% | 84.64% | 29.39% |
| | 10 | 377.28 | 75.29 | 0.81 | 0.97 | 2.93 | 301.02 | 19.95% | 0.26% | 79.79% | 27.69% |
| | 11 | 390.78 | 41.47 | 0.45 | 2.44 | 3.03 | 346.88 | 10.61% | 0.62% | 88.77% | 14.87% |
| | 12 | 634.74 | 95.29 | 0.63 | 1.51 | 3.20 | 537.95 | 15.01% | 0.24% | 84.75% | 19.72% |
| | | | | | | | | | | | |
| blood contamination | 13 | 427.37 | 13.59 | 0.51 | 18.12 | 2.94 | 395.66 | 3.18% | 4.24% | 92.58% | 17.24% |
| | 14 | 544.35 | 56.53 | 0.73 | 16.43 | 3.00 | 471.39 | 10.38% | 3.02% | 86.60% | 24.44% |
| | 15 | 651.93 | 77.39 | 0.68 | 10.58 | 3.29 | 563.96 | 11.87% | 1.62% | 86.51% | 20.55% |
| | 16 | 345.96 | 56.30 | 0.56 | 1.85 | 2.92 | 287.80 | 16.27% | 0.54% | 83.19% | 19.31% |
| | 17 | 356.86 | 33.50 | 0.56 | 13.28 | 2.92 | 310.09 | 9.39% | 3.72% | 86.89% | 19.31% |
| | 18 | 307.26 | 13.37 | 0.65 | 19.93 | 2.89 | 273.96 | 4.35% | 6.49% | 89.16% | 22.66% |
| | 19 | 436.46 | 83.27 | 0.95 | 13.85 | 2.31 | 339.35 | 19.08% | 3.17% | 77.75% | 40.98% |
| | 20 | 542.77 | 26.74 | 0.60 | 26.74 | 3.53 | 489.28 | 4.93% | 4.93% | 90.15% | 16.93% |
| | 21 | 456.04 | 38.40 | 0.50 | 27.05 | 3.41 | 390.59 | 8.42% | 5.93% | 85.65% | 14.57% |
| | 22 | 462.77 | 60.69 | 0.58 | 45.53 | 3.62 | 356.55 | 13.11% | 9.84% | 77.05% | 15.89% |
| | 23 | 615.37 | 58.07 | 0.51 | 44.86 | 3.21 | 512.44 | 9.44% | 7.29% | 83.27% | 15.79% |
| | 24 | 481.75 | 37.02 | 0.77 | 29.69 | 3.48 | 415.04 | 7.68% | 6.16% | 86.15% | 22.01% |

*Table 2.* Data values for both uncontaminated and contaminated blood sample groups.